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Efficacy in cats of a novel topical combination of fipronil, (S)-methoprene, eprinomectin, praziquantel, against induced infestations of *Echinococcus multilocularis*

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ABSTRACT

Although foxes are the main reservoir of *Echinococcus multilocularis*, it is recognized that dogs and cats also may become infected. In cats the infection and egg production rates are usually low. Nevertheless, cats are a potential source of transmission of *E. multilocularis*. Due to the high human medical significance of *E. multilocularis* infection, it is important in endemic areas that owned cats are dewormed regularly. This paper presents the efficacy results of a new topical formulation, Broadline® (Merial) tested against *E. multilocularis* infection in cats. Two blinded laboratory studies were conducted to evaluate this novel topical combination of fipronil, (S)-methoprene, eprinomectin, and praziquantel against *E. multilocularis*. In each study, purpose-bred cats were assigned randomly to two treatment groups of 10 cats each: one untreated control group and one group treated at the minimum therapeutic dose of 0.12 mL/kg bodyweight to deliver 10 mg fipronil, 12 mg (S)-methoprene, 0.5 mg eprinomectin and 10 mg praziquantel/kg bodyweight. The cats were inoculated orally with *E. multilocularis* protoscolices, 22 or 23 days before treatment. Based on necropsy and intestinal worm count, 8 or 11 days after treatment, the two studies confirmed 100% efficacy of Broadline® against adult *E. multilocularis*.

1. Introduction

Echinococcus multilocularis is distributed widely in the Northern Hemisphere and is endemic in central Europe and in various regions of North America and Asia (Gottstein et al., 1996; Romig, 2003; Romig et al., 2005; Davidson et al., 2012). The definitive host of *E. multilocularis* is the fox (red fox and arctic fox), but other carnivores, such as wild canids (e.g. wolves, coyotes), wild felids, raccoons, or domestic dogs and cats, may serve as hosts. Intermediate hosts are small rodents, mainly *Microtidae* (e.g. voles, muskrats, lemmings, hamsters, gerbils and related species) (Eckert and Deplazes, 2004; Burlet et al., 2011). The prevalence of the parasite is considered to be increasing and it is spreading to new countries through the movement of wildlife. The parasite also has spread from rural to urban areas, because of the increase in urban fox populations (Romig, 2003; Deplazes et al., 2004; Jenkins et al., 2005; Fischer et al., 2005; Deplazes, 2006; Takumi et al., 2008; Dakkak, 2010; Siko et al., 2011; Davidson et al., 2012; Takumi et al., 2012). Human exposure is becoming more common in endemic area and may be exacerbated by contact with infested domesticated carnivores (Dyachenko et al., 2008). *E. multilocularis* has a high human medical significance by causing

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alveolar echinococcosis, which can be fatal (Vuitton et al., 2003; Deplazes and Eckert, 2001).

Transmission of *E. multilocularis* usually occurs via a sylvatic cycle, with the possibility of infection of domestic dogs and cats via infected small mammals. The definitive host harbours the adult cestode, and the infestation typically is asymptomatic. Eggs are passed in the faeces of the definitive host and contaminate the environment. Eggs have a patency of 2–4 months and may remain infective in the environment for many months or years, in cool and damp conditions. Eggs are ingested by an intermediate host and develop into alveolar metacestode cysts. Metacestode cysts contain numerous vesicles, each containing high numbers of protoscolices, the infective form. In the intermediate host, the metacestode larva is characterized by an exogenous tumour-like tissue proliferation, which progressively infiltrates the infected organ and may lead to severe disease and death. Intermediate hosts are preyed upon by carnivores. When ingested by the definitive host, protoscolices evolve into adult *E. multilocularis* in the small intestine, and egg production can begin as early as 25 days after infestation. Eggs also may be ingested by aberrant hosts, such as pigs, horses, monkeys and humans. Such ingestion may cause alveolar echinococcosis, one of the most lethal helminthic infection in humans (Deplazes and Eckert, 2001; Bowman et al., 2003; Eckert and Deplazes, 2004; Davidson et al., 2012).

In comparison to dogs and wild canids, cats shed fewer *E. multilocularis* eggs, for a shorter duration. Therefore, cats appear to have a lesser role in the maintenance of *E. multilocularis* in endemic area, and infestations in cats may be of more limited public health significance (Thompson et al., 2003, 2006; Kapel et al., 2006; Learmount et al., 2012). Nevertheless, cats are regarded as a potential source of contamination for intermediate hosts including humans (Thompson et al., 2003; Dyachenko et al., 2008) and should be treated on a regular basis when they have regular outdoor access in endemic areas. Praziquantel, administered by topical and oral routes, has long been established as an efficient treatment against *E. multilocularis* infestations in cats and dogs (Jenkins and Romig, 2000; Charles et al., 2005; Schroeder et al., 2009; Knaus et al., 2014; Rehbein et al., 2014). Merial has developed Broadline®,[1] a topical endo- and ectoparasiticide drug for cats combining fipronil 8.3% (w/v), (S)-methoprene 10% (w/v), eprinomectin 0.4% (w/v), and praziquantel 8.3% (w/v). The present investigations were carried out to evaluate the efficacy of a single topical treatment with that novel formulation against *E. multilocularis* infestations in cats.

2. Materials and methods

The two study designs used were in accordance with the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products VICH GL7, "Efficacy of Anthelmintics: General Requirements" (Vercruysse et al., 2001) and VICH GL20 "Efficacy of Anthelmintics: Specific Recommendations for Felines" (Vercruysse et al., 2002), and the "World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anthelmintics for dogs and cats" (Jacobs et al., 1994); and the studies were conducted in compliance to VICH GL9 entitled *Good Clinical Practice*. Animals were managed similarly and with due regard for their well-being in compliance with the local Ethics Committee approvals and other local applicable regulations and requirements.

Table 1
Characteristics of experimental animals.

Study	Sex[a]	Age[b] (months)	Pre-treatment[c] body weight (kg)
Study 1	10M, 10 F	7	2.4–5.8
Study 2	10M, 10 F	7–11	2.3–6.1

[a] M, male; F, female.
[b] Age on the day of treatment (=Day 0).
[c] Day −2, prior to treatment.

The studies described here were blinded, laboratory-based, single centre, clinical efficacy studies, with an untreated control group and used a randomized block design based on bodyweight. Healthy laboratory purpose-bred European Short Hair cats were included (see Table 1). None of the cats had been treated with an endoparasiticide within 3 months of acclimatization start.

In the two studies, cats were housed individually in a controlled environment with an air conditioning system. They were identified individually with microchips. They were observed at least once daily for general health during the entire in-life phase and had a veterinary examination for suitability before *E. multilocularis* inoculation. The cats were also observed hourly four times after application of the treatment.

After acclimatization of at least 8 days to the study environment and conditions, cats were sedated and orally inoculated with protoscolices. Cats were fasted overnight before the inoculation. A viability assessment, based on motility of protoscolices seen under the microscope, was performed shortly before the inoculation. For each inoculation, the container of *E. multilocularis* was shaken lightly to ensure uniform suspension and the volume loaded in a disposable syringe. The syringe was connected to an appropriate-sized flexible tube, the tube was inserted into the oesophagus, and the inoculum expelled. The tube was flushed with ~2 mL of saline before removal. Each cat was inoculated with approximately 30,000 (Study 1) and 38,000 protoscolices (Study 2) at Day −23 (Study 1) or Day −22 (Study 2). Protoscolices of *E. multilocularis* for both studies originated from common voles infected with recent field isolates (<10 years) that were obtained from red foxes from central Europe (Germany).

Cats were ranked by body weights and then assigned to 10 blocks of 2 cats each. Within blocks cats were allocated randomly to the untreated control group or the treated group. All cats were weighed on study Day −2 for treatment dose calculation. Treatments were applied on study Day 0. The test product was applied at the minimum therapeutic dose of 0.12 mL/kg body weight directly on the skin in the midline of the neck between

[1] Broadline® is a trademark of Merial; all other marks are the property of their respective owners.

the base of the skull and the shoulder blades in a single spot. The dosage was 10 mg/kg fipronil, 12 mg/kg (S)-methoprene, 0.5 mg/kg eprinomectin, and 10 mg/kg praziquantel. Timing of the interval between inoculation with *E. multilocularis* and treatment was designed so that the parasites were expected to be adults on study Day 0. All cats were observed hourly four times after treatment. On study Day 8 (Study 1) or study Day 11 (Study 2), cats were humanely euthanized, and the intestines were collected for parasite identification and count to assess treatment effectiveness. Intestines were ligated and collected from pyloric to rectal end. They were packaged individually and stored frozen at −70 °C for at least 4 weeks before parasite counts. For the counts, thawed intestines were opened completely with a pair of scissors, and the entire contents (including the mucosa) were screened through a 150 μm mesh size sieve. The remaining material on the sieve was retained and preserved. A 20% aliquot of the preserved material from each complete sample was examined for *E. multilocularis* scolices using a dissection microscope. For samples containing <10 scolices in the 20% aliquot, the remaining 80% were examined for *E. multilocularis* to provide a total count. For samples containing ≥10 scolices, total counts per animal were calculated by multiplying the number of scolices actually counted by the aliquot factor of 5.

Counts of the parasites were transformed to the natural logarithm of (count + 1) for calculation of geometric means for each treatment group. Efficacy was determined for *E. multilocularis* by calculating the percent efficacy as $100[(C-T)/C]$, where C is the geometric mean count among untreated controls and T is the geometric mean count among the treated animals. To compare the geometric means of the two groups, the mean of the log-counts of the treated group was compared to the mean of the log-counts of the untreated control group using an *F*-test adjusted for the allocation blocks used to randomize the animals to the treatment groups. The mixed procedure in SAS® version 9.1.3 was used for the analysis, with the treatment groups listed as a fixed effect and the allocation blocks listed as a random effect. All testing used a two-sided significance level $\alpha = 0.05$.

3. Results

No adverse events or other health problems were observed after treatment application and throughout the studies, indicating that treatment with topical formulation of fipronil, (S)-methoprene, eprinomectin and praziquantel was well accepted.

In Study 1, 2–230 *E. multilocularis* scolices were recovered from 9 untreated control cats, and the geometric mean number for the group was 24.0. In Study 2, 18–5445 *E. multilocularis* scolices were recovered from 8 untreated control cats, and the geometric mean number for the group was 100.8. In both studies, no *E. multilocularis* scolices were recovered from any of the treated cats. In both studies, adequacy of infestation per VICH Guidelines 7 and 20 was achieved, and efficacy was 100% with a significant difference ($P < 0.001$) from the control group (Table 2).

The establishment rate (mean number of *E. multilocularis* scolices found at necropsy/number of protoscolices inoculated in the untreated control groups) of the two *E. multilocularis* isolates were 0.08% for Study 1 and 0.27% for Study 2.

4. Discussion

The life cycle of *E. multilocularis* is predominantly sylvatic with foxes and raccoon dogs as definitive hosts, nevertheless domestic dogs and to a lesser extent cats may take this role. It has been suggested that the significance of cats in its epidemiology is lower than that of foxes or dogs, when comparing the reproductive potential of the parasite in these hosts (Kapel et al., 2006; Thompson et al., 2006). Establishment rates of induced infections in these studies (0.08% or 0.27%) are in line with rates of other studies (Thompson et al., 2006) and complement the known low susceptibility of cats as definitive hosts. However, due to their hunting behaviour, cats may pray on rodents more often than dogs, so that cats with regular outdoor access may be more frequently exposed to protoscolices of *E. multilocularis* than expected. Molecular-biological examination of faecal samples in a cross-sectional survey identified *E. multilocularis* DNA in 68% of taeniid-positive samples from cats in Europe (Dyachenko et al., 2008).

Even though the risk of zoonotic transmission from cats is still poorly understood, in order to prevent any risk of cats acting as a source of eggs, they should be treated on a regular basis when they have outdoors access and hunting activities in endemic areas. Praziquantel is an established compound with well-proven efficacy against cestodes, including *E. multilocularis*, and has an excellent safety profile in several animal species and when given by different routes of administration (Jenkins and Romig,

Table 2
Parasite counts and therapeutic efficacy of Broadline® spot-on against experimentally induced *Echinococcus multilocularis* in cats.

	Echinococcus multilocularis counts				Efficacy (%)[b]	*P*-value[c]
	Untreated (control)		Treated[a]			
	NI/NG[d]	GM[e] (range)	NI/NG	GM (range)		
Study 1	9/10	24.0 (0–230)	0/10	0.0 (0)	100	<0.001
Study 2	8/10	8.33 (0–5445)	0/10	0.0 (0)	100	<0.001

[a] Broadline® spot on = fipronil (8.3%, w/v), (S)-methoprene (10%, w/v), eprinomectin (0.4%, w/v), and praziquantel (8.3%, w/v); at 0.12 mL/kg body weight.
[b] Efficacy = 100 [(geometric mean untreated (control) group – geometric mean Topical FMEP group)/geometric mean untreated (control) group].
[c] Two-sided *p*-value comparing the worm burden of the Topical FMEP group with untreated (control) group.
[d] NI/NG, number of cats infected/number of cats in group.
[e] Geometric mean count (based on transformation to ln[count + 1]).

2000; Charles et al., 2005; Schroeder et al., 2009). Results of these two studies using induced infections of *E. multilocularis* have demonstrated a high level of efficacy of the novel topical combination of fipronil, (S)-methoprene, eprinomectin and praziquantel. These data complement the results of a study which was conducted to evaluate the efficacy of Broadline® against helminth infections under field conditions (Rehbein et al., 2014). The novel topical combination formulation can provide an efficacious and convenient solution for the treatment of cats for infections with *E. multilocularis*.

Conflict of interest

The work reported herein was funded by Merial Limited, GA, USA. All authors are current employees of Merial.

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